progenitors are not able to produce early cell types even in an early environment.

The questions that face us now are still very basic. If retinal cells progress through steps of competence, how many such steps are there, and how are these steps controlled. Another fundamental question is how progenitors know how many cell cycles to go through to generate all the correct fates in just the right numbers and proportions. Several of the basic cell types come in an amazing variety of different subtypes; for example, in the zebrafish retina there are as many as 17 different morphological subtypes of bipolar cells, and probably more than 20 subtypes of amacrine cell. How are all these subtypes generated? Are they molded from of an ancestral fate that they all share, or do they arise from different lineages?

Twenty years on, while Christine largely turned her attention back to axon guidance and the biology of the growth cone after this paper, I was captivated by cell determination, and I have spent the better part of the last several years following up issues raised in this paper.

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An α_{40} Subunit of a GTP-Binding Protein Immunologically Related to G_0 Mediates a Dopamine-Induced Decrease of Ca^{2+} Current in Snail Neurons

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Dopamine induces a decrease in voltage-dependent Ca^{2+} current in identified neurons of the snail *H. aspersa*. This effect is blocked by intracellular injection of activated B. pertussis toxin and of an affinity-purified antibody against the α subunit of bovine G_0. protein. The dopamine effect is mimicked by intracellular injection of mammalian α_0. In snail nervous tissue, pertussis toxin ADP-ribosylates a single protein band on SDS gels, and this band is recognized in immunoblots by the anti-α_0 antibody. We propose that this is a 40 kd subunit of a molluscan G protein immunologically related to α_0 and that it mediates the effect of dopamine on Ca^{2+} currents in identified snail neurons.

The 1980s was an exciting time for the study of neuromodulation and its role in shaping the firing properties of neurons. During this decade, it was slowly becoming clear that, in addition to the well-understood rapid (ms) ionotropic actions of fast transmitters such as glutamate and GABA, completely separate metabotropic pathways exist which are triggered by G protein-coupled receptors and modify the properties of neurons and synapses over much longer times. These actions could lead to neural network reconfigurations, allowing a single network to produce a variety of different behavioral outputs. However, the detailed molecular and biophysical mechanisms mediating these slow modulatory actions were not yet clear. Several second messenger pathways were starting to be understood, including those mediated by cAMP, cGMP, Ca^{2+}, and phosphoinositide metabolism, but these did not explain all the modulatory actions that had been discovered.

In 1986, we were working in the lab of Dr. Hersch Gerschenfeld at the Ecole Normale Supérieure in Paris. "Coco," as his friends called him, and Danièle had deep interests in second messenger mechanisms underlying monoamine and peptide actions on ionic currents. By 1986, Danièle and Coco were focusing their attention on modulation of calcium channels, which play crucial roles in regulating synaptic transmission and cellular firing properties. They had already shown in identified neurons of mollusks, for example, that serotonin enhances I_{Ca} via a cGMP pathway and that the peptide cholecystokinin-8 irreversibly reduces I_{Ca} by a pathway involving PLC and PKC. However, they then discovered an effect that was not easily explained. Dopamine

reversibly reduced I_{Ca} in selected *Helix* neurons, but this mechanism was not mediated by cAMP, cGMP, or calcium metabolism (Paupardin-Tritsch et al., J. Neurosci. *5*, 2522–2532, 1985). Ron joined the group during a sabbatical year from Cornell, and we decided to explore the mechanisms of this dopamine effect.

As we were beginning our work, we read a breakthrough paper from Kathy Dunlap's lab showing, in chick sensory neurons, that the newly discovered G proteins appear to be in the direct pathway for norepinephrine and GABA reductions of I_{Ca} (Holz et al., Nature *319*, 670–672, 1986). These reductions could be blocked by GDP-β-S or by Pertussis toxin (PT), which inactivate the $G_{i/Go}$ family of G proteins, and their actions could be mimicked by GTP-γ-S, which irreversibly activates G proteins. As we were doing our experiments, a paper by Hescheler et al. (Nature *325*, 445–447, 1987) implicated the G_o pathway more directly in N x G cells, where opiate peptides reduce I_{Ca}, by showing that the purified G_o subunit α_o could restore the response to opiate peptides in PT-treated cells,. However, this conclusion was tentative since G_o molecules could be mimicking another, as yet unknown, G protein.

In our *Neuron* paper, we extended this logic by another step. In identified *Helix* neurons, the dopamine-mediated reduction of I_{Ca} could, as expected, be mimicked and occluded by GTP-γ-S and blocked by PT and GDP-β-S. Joel, working at CNRS-INSERM in Montpellier, had been isolating G proteins from bovine brain and generating very specific antibodies to help differentiate the roles of each G protein. He provided us with purified bovine α_o as well as a rabbit anti-α_o antibody that did not recognize any other known G protein and only recognized a single band from snail proteins. As Hescheler et al. had found, injection of activated α_o-GTP-γ-S by itself directly caused a decrease in I_{Ca}. When we injected the anti-α_o antibody (using a third electrode in the large molluscan neurons), it significantly blocked the effect of dopamine. We thus proposed that a snail G protein related to mammalian G_o mediates dopamine's reduction of Ca^{2+} current in identified snail neurons. At that time we did not know whether there was another unidentified second messenger involved or whether α_o acted directly on the Ca^{2+} channel, and we did not pursue this issue.

At about this time, a huge controversy erupted over which G protein subunits might directly affect ion channels without mediation of any second messenger pathways. Using inside-out patches of atrial cells, in a paper in *Science*, Yatani et al. (Science *235*, 207–211, 1987) reported that a Gα subunit could activate potassium channels, while in a *Nature* paper, Logothetis et al. (Nature *325*, 321–326, 1987) argued instead that the Gβγ complex was responsible. Similar arguments were made for direct inhibitory effects of G protein α or βγ subunits on I_{Ca}. All this research was done with proteins biochemically purified from complex neural tissue, which were subject to contamination. This argument lasted for nearly a decade, until experiments using pure recombinant G proteins and directed G protein gene expression showed that it is indeed the Gβγ subunits that directly inhibit calcium channels in almost all systems studied.

It was thrilling to do these experiments, which contributed to the rapidly growing G protein saga. We share wonderful memories of working with Coco Gerschenfeld, whose enthusiasm for these experiments was as strong as the cigars he loved to smoke. His death in 2004 brought to an end a magnificent career in neuroscience.

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